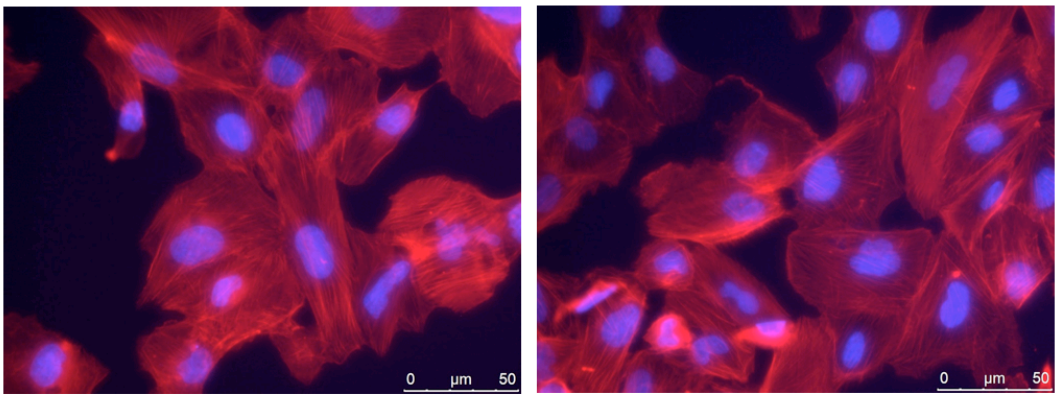
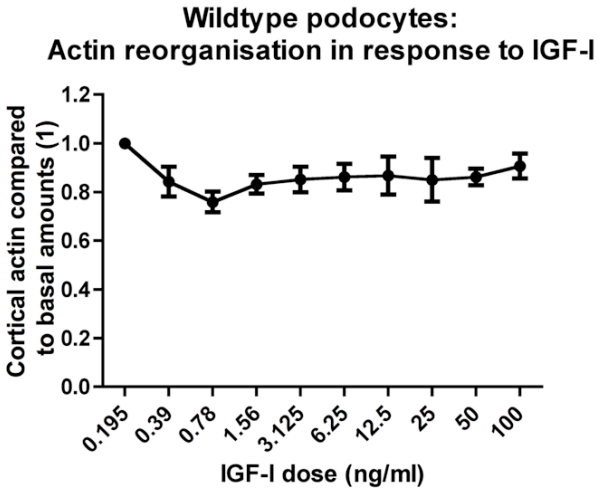
Figure S1

A

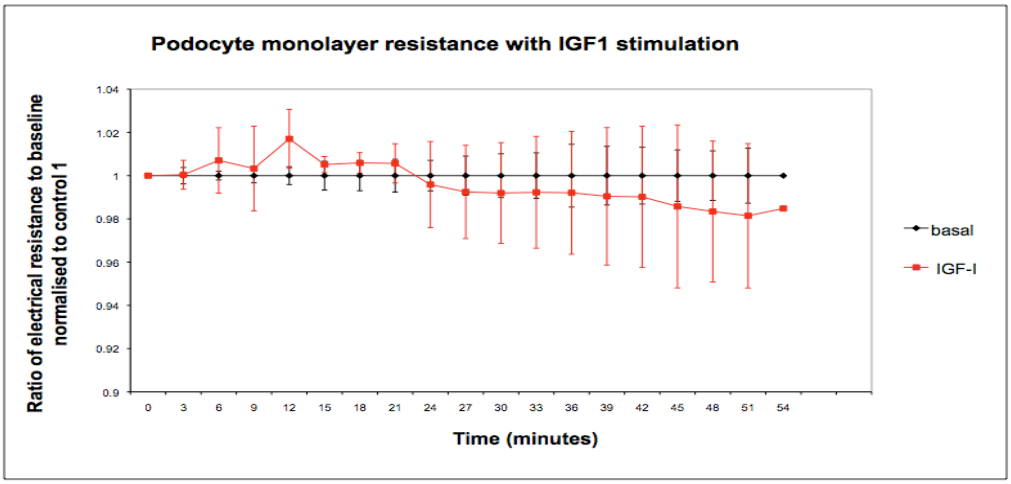


BASAL 100ng/ml IGF1

B



C



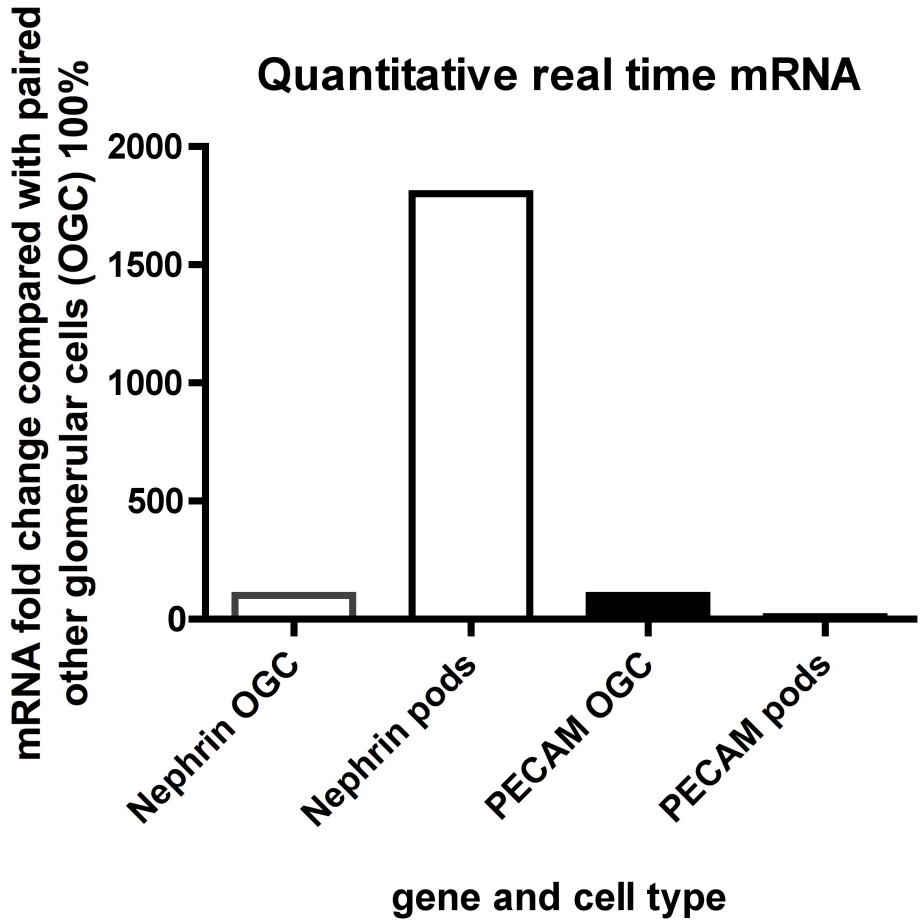
IGF-1 does not remodel the actin cytoskelton of conditionally immortalized human podocytes.

A IF for phalloidin (red). Cells exposed to IGF1 for 15 minutes

B There is no objective difference in actin remodelling using the in cell analyzer **B** (n=8 SEM shown) 15 minute stimulation.

C ECIS shows IGF1 has no effect on monolayer electrical resistance when compared with basal conditions. 100ng/ml IGF1 n=4

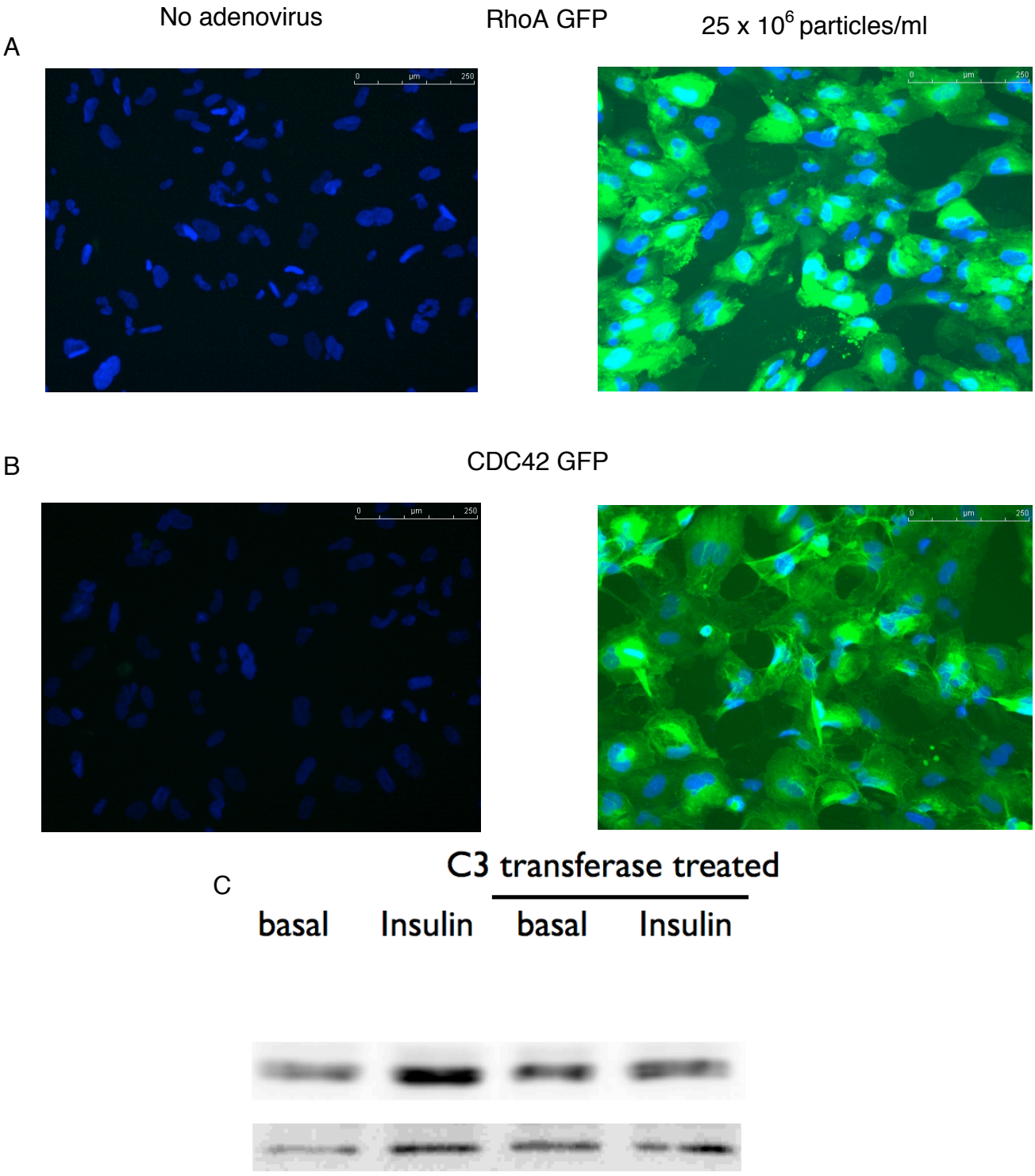
Figure S2



FACS sorting of glomerular cells showing CFP positive fraction are predominantly podocytes.

CFP positive fraction have 18 times more nephrin and 10 times less PECAM mRNA signal compared to the CFP negative fraction as measured by quantitative real time PCR.

Figure S3

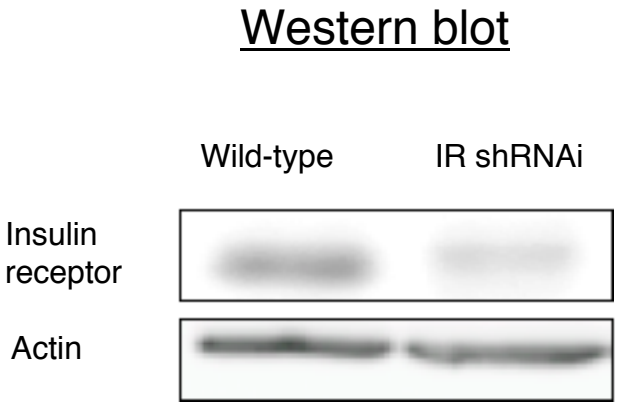


Dominant negative adenoviral transfection of differentiated ciPods.

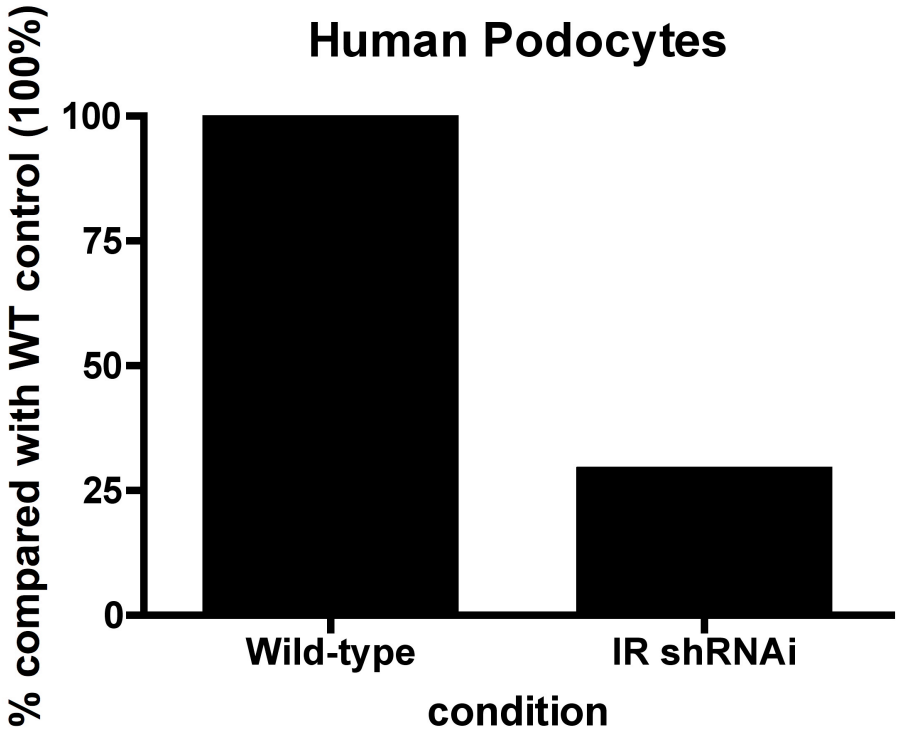
A. RhoA GFP construct was transfected into differentiated cells and cellular expression was checked at 4 hours by fluorescent microscopy (Green). Excellent transfection efficiency detected at titre of $25x10^6$ particles /ml. Cellular nuclei shown with DAPI (blue)
B. Same viral titre worked for CDC42 GFP construct.
C. 5 minute insulin stimulation of non-transfected (left 2 lanes) and Rho dominant negative (C3 transferase) at $25x10^6$ particles /ml. Loss of insulin induced GTP activation.

Figure S4

A



B



Lentiviral short hairpin siRNA knockdown of the insulin receptor in immortalized human podocytes.

A. Western blot with equal loading as shown by actin. **B** Real time PCR analysis of human podocytes. This was achieved using the same technique as used for the CFP isolated mouse podocytes.

Table S1

Primer target	Forward primer	Reverse primer
Insulin receptor	GATGTGCACCCCATGTCTG	ATACCAGAGCATAGGAG
IGF-1 receptor	GTGGGGGCTCGTGTTTCTC	GATCACCGTGCAGTTTTCCA
Nephrin	CCACTGGGGCTGAAGGTTGT	TCAGCCCAGTCAGTGTGAAG
PECAM	CAAGCAAAGCAGTGAAGCTG	AGCAGGACAGGTCCAACAAC
HPRT (house keeping)	GGCTATAAGTTCTTTGCTGACCTG	AACTTTTATGTCCCCCGTTGA

Primer sequences used in the mouse glomerular cell CFP sorting experiments.

Supplementary Experimental procedures

Mouse glomerular scoring

Mouse glomeruli were scored for severity of matrix accumulation, glomerulosclerosis and mesangial hypercellularity by light microscopy. PAS-stained sections were examined by an experienced nephropathologist (AMH) blinded to experimental conditions. All glomerular profiles (approximately 60 to 100) in a single section were scored for severity of mesangial matrix increase. Each glomerulus was given a score of 0 (normal), 1 (mild, mesangial matrix (MM) increase approximately 2 times the width of a mesangial cell nucleus), 2 (moderate, MM increase approximately 3 to 4 times the width of a mesangial cell nucleus), or 3 (severe, MM increase >4 times the width of a mesangial cell nucleus). The mean glomerular MM score was then calculated for each animal.

Glomerulosclerosis was measured using the 4 quadrant technique (Oudit et al., 2006) and mesangial hypercellularity using the principles described recently by Roberts et al (Roberts et al., 2009)

REFERENCES

Oudit, G.Y., Herzenberg, A.M., Kassiri, Z., Wong, D., Reich, H., Khokha, R., Crackower, M.A., Backx, P.H., Penninger, J.M., and Scholey, J.W. (2006). Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis. Am J Pathol *168*, 1808-1820.

Roberts, I.S., Cook, H.T., Troyanov, S., Alpers, C.E., Amore, A., Barratt, J., Berthoux, F., Bonsib, S., Bruijn, J.A., Cattran, D.C.*, et al.* (2009). The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. Kidney Int *76*, 546-556.